AD _____

Effects of Immunomodulatory Drugs on T lymphocyte Activation and Function

Annual Report

Constantine D. Tsoukas, Ph.D.

June 15, 1989

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-86-C-6166

Research Institute of Scripps Clinic
Department of Molecular and Experimental Medicine
10666 North Torrey Pines Road
La Jolla, California, 92037

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp Date Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Research Institute of Scripps Clinic, Dep.Molec.& Exp. Med.	6b. OFFICE SYMBOL (If applicable)
6c. ADDRESS (City, State, and ZIP Code) 10666 North Torrey Pines Road La Jolla, California 92037	7a. NAME OF MONITORING ORGANIZATION 7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Developm. Command	8b. OFFICE SYMBOL (If applicable)
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-86-C-6166
	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO.
62770A	3M16 2770A871	AB	366

11. TITLE (Include Security Classification)

Effects of Immunomodulatory Drugs on T lymphocyte Activation and Function

12. PERSONAL AUTHOR(S)

Tsoukas, Constantine Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 5/15/88 TO 5/14/89	1989 June 15	15

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	15		Immunomodulatory drugs, proliferation of T cells, IL-1, IL-2, IL-2 receptors, cytotoxic T cells,
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During this third reporting year (5/15/88 to 5/14/89), the following drugs were tested for their immunomodulatory actions: Acridine trihydrochloride (CL246), OK432, WY18251, poly ICLC (AVS1761), Quinolinamine (AVS1300), and Ampligen (AVS2149). These drugs were tested for their effects on the production of antibody that is stimulated upon addition of Pokeweed Mitogen (PWM) to lymphocyte cultures or upon infection of lymphocytes with Epstein-Barr Virus (EBV). The drugs were also tested for their effects on lymphocyte proliferation stimulated by EBV. Finally, the drugs were tested for their ability to modulate the generation of lymphokine activated killer cells (LAK) which are stimulated upon culturing T lymphocytes with Interleukin 2 (IL2). We found that Acridine trihydrochloride had a pronounced inhibitory effect on all of the above tested functions. This inhibition was most pronounced with drug concentrations above 0.1 µg/ml. Quinolinamine at concentrations above 0.1 µg/ml stimulated both the PWM-stimulated IgM and IgG production, as well as the EBV-induced proliferation of lymphocytes. This drug had also a small, but significant augmenting effect on EBV-induced IgM production at >1 µg/ml without, however, affecting the virus-induced IgG production. Finally, at low concentrations (0.001-1 µg/ml) Quinolinamine stimulated the induction of LAK cells while at higher concentrations (2.5-20 µg/ml) inhibited their production. From the rest of the drugs poly ICLC inhibited the EBV-stimulated production of IgM antibody and the generation of LAK cells, WY18251 and OK432 had a small inhibitory effect on EBV-stimulated IgM production and the rest of the drugs exhibited neither inhibition nor stimulation in the assays studied.

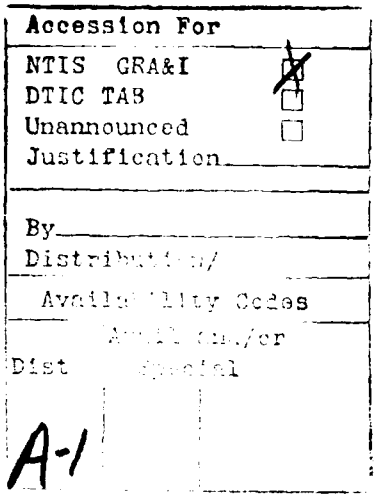
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia Miller	22b. TELEPHONE (Include Area Code) (301) 663-7325
	22c. OFFICE SYMBOL SGRD-RMI-S

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CFR46



BODY OF REPORT

During this third reporting year (5/15/88 to 5/14/89), the following drugs were tested for their immunomodulatory actions: Acridine trihydrochloride (CL246), OK432, WY18251, poly ICLC (AVS1761), Quinolinamine (AVS1300), and Ampligen (AVS2149). These drugs were tested for their effects on the production of antibody that is stimulated upon addition of Pokeweed Mitogen (PWM) to lymphocyte cultures or upon infection of lymphocytes with Epstein-Barr Virus (EBV). The drugs were also tested for their effects on lymphocyte proliferation stimulated by EBV. Finally, the drugs were tested for their ability to modulate the generation of lymphokine activated killer cells (LAK) which are stimulated upon culturing T lymphocytes with Interleukin 2 (IL2).

In order to study effects on mitogen-stimulated antibody production, we employed the Pokeweed Mitogen (PWM)-driven system where peripheral blood lymphocytes are stimulated *in vitro* and the produced antibody is measured in the tissue culture supernatant by ELISA. In particular, duplicate 12x75 mm tissue culture tubes containing $1x10^6$ peripheral blood lymphocytes in 1 ml of RPMI 1640-10% FCS and an optimal amount of PWM (in our experiments 0.1 µg/ml) are incubated for 9 days in a humidified atmosphere of 95% air-5% CO_2 at 37^0C. The culture supernatants are collected, cleared of any cells by centrifugation and their IgM and IgG antibody content tested by ELISA. In the ELISA assay duplicate microtiter plate wells are pre-coated with either goat anti-human IgM or IgG (1 µg/ml), the samples are added and then developed with a pre-determined optimal concentration of Alkaline Phosphatase (AP)-conjugated goat anti-human IgM or IgG respectively in the presence of substrate. The amount of antibody in each sample is then quantified by assessing the amount of colored end-product by optical density scanning of the plate at 405 nm.

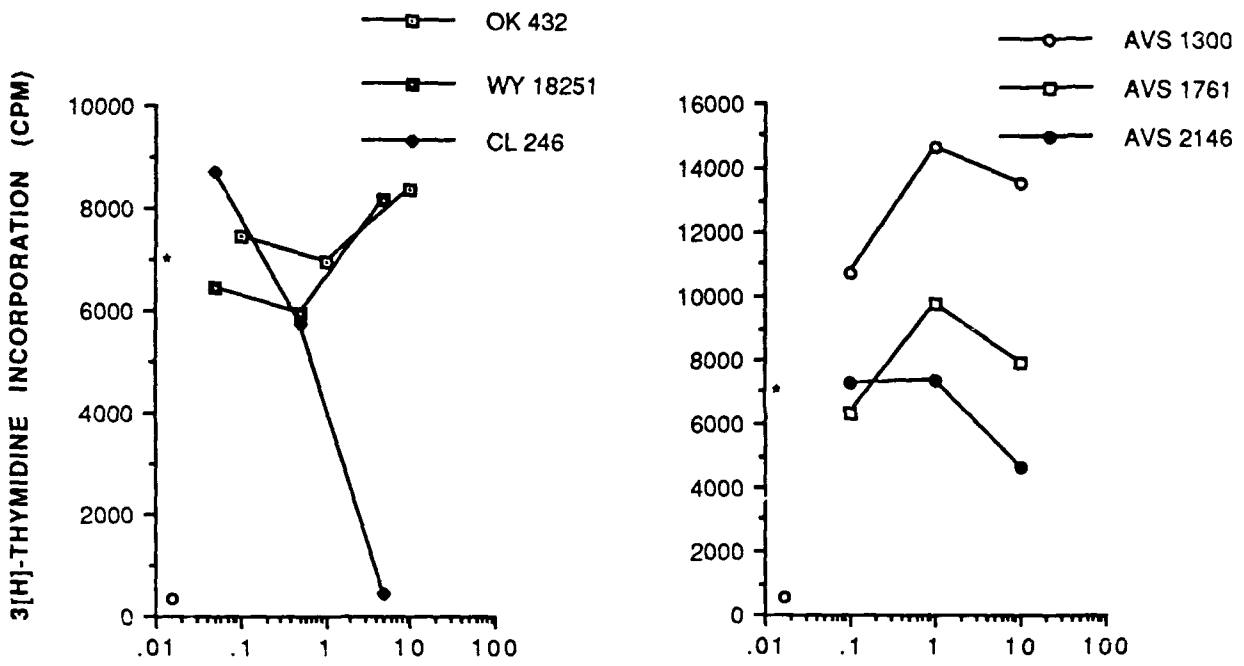
To test the effects of the drugs, peripheral blood lymphocytes were incubated with various concentrations of each drug and 0.1 µg/ml PWM or medium as a control. The secreted IgM and IgG was then quantified by ELISA. The results shown in Figure 1 are representative of four experiments using different blood donors. It can be seen that Acridine trihydrochloride has a dose dependent suppressive effect for both antibody isotypes. In contrast, Quinolinamine had a stimulatory effect for IgM and IgG. The rest of the drugs had no significant effects on antibody production as tested in this system. None of the drugs had toxic effects in the concentration range tested.

In the above experiments, PWM induces an antibody response which is dependent on the participation of T cells thus, any effects of the drugs could be interpreted either as effects on T or B lymphocytes. In the experiments described in this section the use of EBV addresses this question. EBV is a specific B lymphotropic virus which infects B cells and causes their proliferation and production of antibody. Thus, any effects of the drugs in the EBV system must be directed specifically to the B cell itself. In this system, human peripheral blood lymphocytes are purified by removing T cells with sheep red blood cell rosetting and monocytes by adherence on plastic dishes. This gives a population of B cells which is >90% surface Ig^+ and contains <1% T cells. The rest of the cells in the population are residual monocytes and some natural killer cells. This degree of purity is sufficient for the studies, considering that EBV specifically infects B cells only. The B cells are placed in culture dishes and incubated in the presence of an optimal viral dose and various drug concentrations. Appropriate positive and negative controls are also included. After an appropriate incubation period cellular proliferation is assessed and the production of antibody released in the culture supernatant is quantified by ELISA.

When the effects on EBV-induced proliferation were assessed, we found that Acridine trihydrochloride was inhibitory at concentrations >5µg/ml while Quinolinamine was stimulatory at concentrations >0.1 µg/ml (Figure 2). As for the effects on the EBV-induced antibody production, Acridine trihydrochloride again displayed a suppressive effect at similar concentrations while Quinolinamine stimulated only the IgM response, but not the IgG response (Figure 3). WY18251 and OK432 had a slight inhibitory effect on the IgM production. (Figure 3). The rest of the drugs had no detectable effects at the concentrations tested. The results with Acridine trihydrochloride and Quinolinamine are very consistent with those obtained with PWM, suggesting that the effects of these drugs are directed against B lymphocytes.

APPENDIX

FIGURE 2



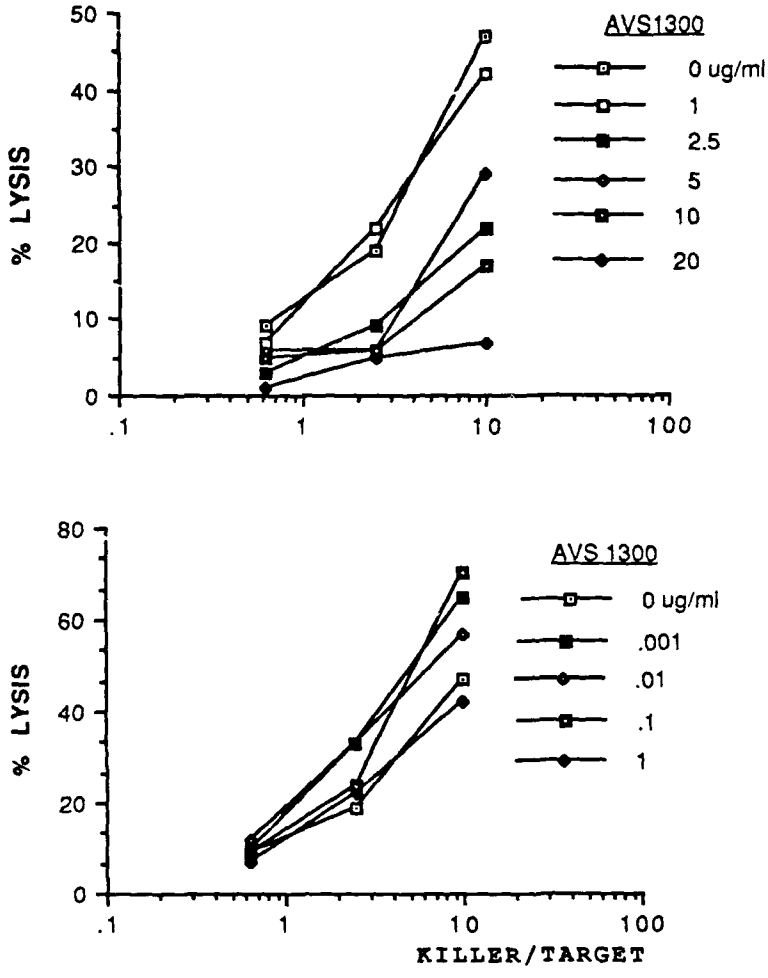
DRUG CONCENTRATION (µg/ml)

Effects of drugs on the EBV-induced proliferation of B lymphocytes: The B lymphocytes were obtained by depleting peripheral blood lymphocytes of T cells and macrophages with sheep red blood cell rosetting and plastic adherence respectively. B cells, $2x10^5$ per microtiter tray well, were cultured in total volume 0.2 mls for 8 days with or without Epstein-Barr virus (an 1:5 dilution of culture supernatant of the EBV-producer marmoset cell line B95-8) in the presence of various drug concentrations as indicated above. Cultures were incubated for 8 days and proliferation was assessed by addition of 3[H]-thymidine (1 µCi/well) during the last 5 hours of incubation. The "*" indicates the positive control with EBV without any drug and the "o" indicates the negative control with only medium. The results are expressed as average CPM of triplicate cultures versus drug concentration. The standard deviation of replicate points was <10% of the average. The results are typical of four separate experiments using different donors.

Legend to Figure 3

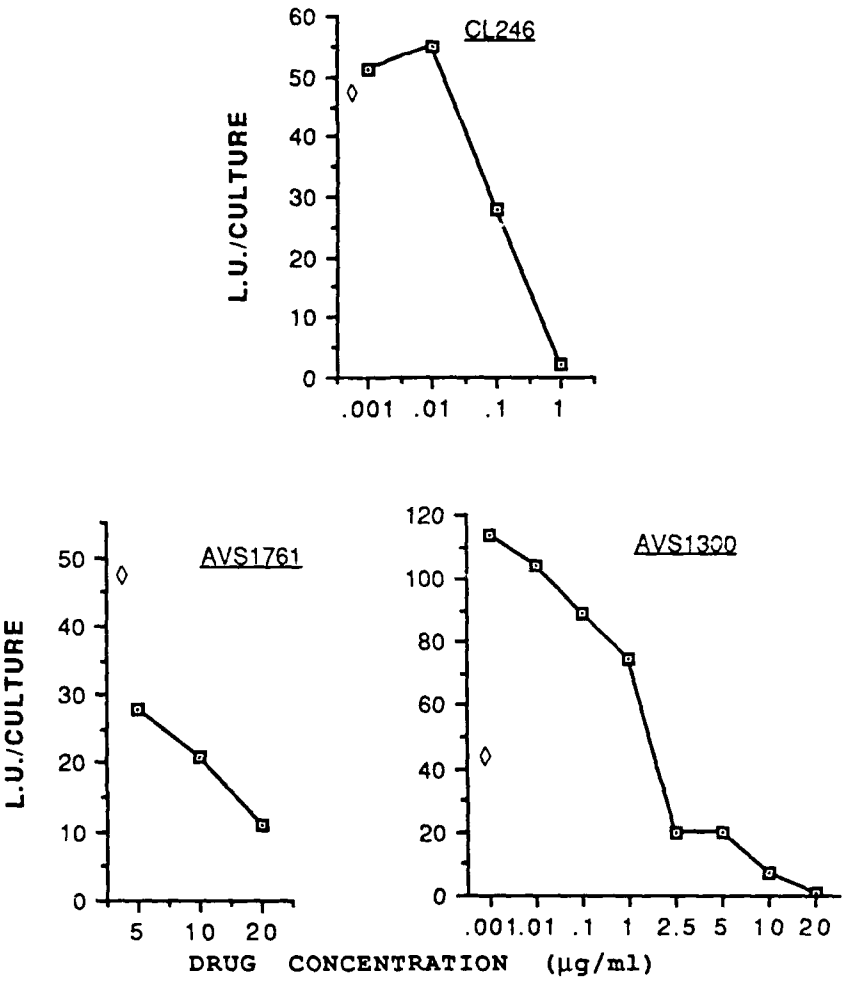
Effects of Drugs on the EBV-induced IgM and IgG production by B lymphocytes: Peripheral B lymphocytes were isolated as described in the legend of the figure above and cultured at $1x10^6$ cells per well per ml with or without EBV (1:5 dilution of virus-containing supernatant) in the presence of various drug concentrations as indicated above. After 14 days of culture the cells were removed and the supernatants were assayed for the presence of IgM and IgG using a solid phase ELISA assay described in the text above. The "*" indicates the positive control with EBV without any drug and the "o" indicates the negative control with only medium. The results are expressed as average O.D. at 405 nm of duplicate determinations versus drug concentration. The standard deviation of replicate points was <5% of the average. The results are typical of four separate experiments using different donors.

FIGURE 5



See legend of Figure 4. Results are representative of three replicate studies.

FIGURE 7



The results presented in Figures 4 and 5 have been analyzed in terms of Lytic Units (L.U.) per culture. One L.U. in this case is defined as the amount of LAK cells causing 20% lysis of the targets. The L.U. per culture were calculated from the total number of viable LAK cells recovered in each culture. The cell viability in all cultures ranged from 80-95%. The (◊) represents the control culture without any drug.